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BRIEF REPORT

Rapid and Effective Virucidal Activity of Povidone-Iodine Products Against Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Modified Vaccinia Virus Ankara (MVA)

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ABSTRACT

Introduction: Since the first case of Middle East Respiratory Syndrome coronavirus (MERS-CoV) infection was reported in 2012, the virus has infected more than 1300 individuals in 26 countries, and caused more than 480 deaths. Human-to-human transmission requires close contact, and has typically occurred in the healthcare setting. Improved global awareness, together with improved hygiene practices in healthcare facilities, has been highlighted as key strategies in controlling the spread of MERS-CoV. This study tested the in vitro efficacy of three formulations of povidone iodine (PVP-I: 4% PVP-I skin cleanser, 7.5% PVP-I surgical scrub, and 1% PVP-I gargle/mouthwash) against a reference virus (Modified vaccinia virus Ankara, MVA) and MERS-CoV.

Methods: According to EN14476, a standard suspension test was used to assess virucidal activity against MVA and large volume plating was used for MERS-CoV. All products were tested under clean (0.3 g/L bovine serum albumin, BSA) and dirty conditions (3.0 g/L BSA + 3.0 mL/L erythrocytes), with application times of 15, 30, and 60 s for MVA, and 15 s for MERS-CoV. The products were tested undiluted, 1:10 and 1:100 diluted against MVA, and undiluted against MERS-CoV.

Results: A reduction in virus titer of $\geq$4 $\log_{10}$ (corresponding to an inactivation of $\geq$99.99%) was regarded as evidence of virucidal activity. This was achieved versus MVA and MERS-CoV, under both clean and dirty conditions, within 15 s of application of each undiluted PVP-I product.

Conclusion: These data indicate that PVP-I-based hand wash products for potentially contaminated skin, and PVP-I gargle/mouthwash for reduction of viral load

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in the oral cavity and the oropharynx, may help to support hygiene measures to prevent transmission of MERS-CoV.
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INTRODUCTION

Four of the six coronaviruses (CoVs) that have made the transition from mammalian/avian hosts to humans are endemic in the human population, and typically associated with mild, self-limiting respiratory illness [1]. However, the remaining two human CoVs cause severe respiratory syndromes and are associated with considerable mortality [1]. In 2003, the severe acute respiratory syndrome (SARS)-CoV caused a disease outbreak that claimed nearly 800 lives [2], and for the second consecutive decade this century, a new human CoV has emerged. The Middle East Respiratory Syndrome (MERS)-CoV was first isolated from a 60-year-old man in Saudi Arabia in June 2012 [3]. Three years later, it has been responsible for the infection of more than 1300 individuals in 26 countries, and more than 480 related deaths [4].

Of all the cases of MERS-CoV reported to date, three quarters have occurred within the source country of Saudi Arabia (Table 1) [4]. Aside from a moderate outbreak in the United Arab Emirates (UAE), travel-associated spread to other countries in the Middle East, as well as examples in Europe, North America, Africa and Asia, has typically resulted in very minimal local outbreaks. The clear exception to this is situation in the Republic of Korea, where over 180 cases have been reported, all during 2015 [4]. Infection in the index case followed recent travel to Saudi Arabia, Qatar, UAE and Bahrain [5]. An International Health Regulations Emergency Committee has highlighted five main factors contributing to the spread of MERS-CoV in the Republic of Korea [6]. Briefly, these were (1) a lack of awareness among healthcare workers and the general public; (2) suboptimal infection prevention and control measures in hospitals; (3) crowded emergency rooms and multi-bed hospital rooms; (4) the practice of patients seeking care at multiple hospitals; (5) multiple visitors staying with infected patients in hospital rooms.

Overall, clinical experience with MERS-CoV indicates that its spread within the human population requires close contact; the majority of cases have resulted from human-to-human transmission in healthcare settings [7]. There is good potential for outbreaks to be contained, given suitable levels of awareness and hygiene. The latest outbreak in Korea, however, is testament to the cost of neglecting these basic requirements. A recent study by our group demonstrated impressive, rapid virucidal activity of povidone iodine (PVP-I) against the Ebola virus (EBOV) [8]. PVP-I was also effective against the European reference virus (Modified vaccinia virus Ankara; MVA), which was determined to be a suitable surrogate test agent, facilitating the safe testing of the virucidal activity of antiseptic products against hazardous pathogens, including enveloped viruses such as EBOV [8]. PVP-I is a broad-spectrum antimicrobial, used globally in the medical field—including the Middle East—as a disinfectant for skin, hands and mucosal surfaces as well as for wound treatment and eye applications [9].

Table 1 Number of laboratory-confirmed cases of MERS-CoV reported to WHO, by country and year

Country	2012	2013	2014	2015	Total
Middle East					
Saudi Arabia	5	136	679	217	1037
United Arab Emirates	0	12	57	7	76
Qatar	0	7	2	4	13
Jordan	2	0	10	0	12
Oman	0	1	1	4	6
Iran	0	0	5	1	6
Kuwait	0	2	1	0	3
Yemen	0	0	1	0	1
Total	7	158	756	233	1154
Southeast Asia					
Republic of Korea	0	0	0	185	185
Philippines	0	0	0	2	2
China	0	0	0	1	1
Thailand	0	0	0	1	1
Malaysia	0	0	1	0	1
Total	0	0	1	189	190
Europe					
United Kingdom	1	3	0	0	4
Germany	1	1	0	1	3
The Netherlands	0	0	2	0	2
France	0	2	0	0	2
Austria	0	0	1	0	1
Greece	0	0	1	0	1
Italy	0	1	0	0	1
Total	2	7	4	1	14
Mediterranean and Arab countries					
Tunisia	0	3	0	0	3
Algeria	0	0	2	0	2
Egypt	0	0	1	0	1
Lebanon	0	0	1	0	1
Turkey	0	0	1	0	1
Total	0	3	5	0	8

Table 1 continued

Country	2012	2013	2014	2015	Total
North America					
United States of America	0	0	2	0	2
Total	0	0	2	0	2
Total	9	168	768	423	1368

Data as of 7 July 2015 [4]
MERS-CoV Middle East respiratory syndrome coronavirus, *WHO* World Health Organization

It may be hypothesized that PVP-I would also demonstrate effective disinfectant properties against MERS-CoV. The study reported here evaluated skin cleanser, surgical scrub and gargle/mouthwash formulations of PVP-I for virucidal activity against both the reference virus MVA and MERS-CoV itself.

METHODS

Virucidal Products Tested

Three PVP-I antiseptic products were tested in this study: 4% PVP-I skin cleanser, 7.5% PVP-I surgical scrub and 1% PVP-I gargle/mouthwash, each with the brand name Betadine, manufactured by Mundipharma (Limburg, Germany). This article does not contain any new studies with human or animal subjects performed by any of the authors.

Propagation of the Test Virus

MVA

Methodology for propagation of MVA was as described in [8]. Briefly, baby hamster kidney cells (BHK)-21 cells (cell bank of Friedrich-Loeffler-Institute, Germany) were infected with MVA (Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Germany) and cultured at 37 °C in a humid atmosphere under 5.0% CO_2. The virus was cultivated from confluent monolayers with a maximum age of 2 days.

MERS-CoV

Cultivation of MERS-CoV was based on the same overall method as for MVA. The MERS-CoV, HCoV-EMC/2012 (Erasmus Medical Center, Rotterdam, The Netherlands) was used as the test virus. Vero E6 cells (American Type Culture Collection, ATCC) were used for virus cultivation and the suspension test.

Inactivation Assay

Tests were carried out once in accordance with EN14476:2013/FprA1:2015 at 20 ± 1 °C [10]. The test assay comprised 100 µL virus suspension, 100 µL interfering substance (clean, 0.3 g/L bovine serum albumin [BSA] or dirty, 3.0 g/L BSA + 3.0 mL/L erythrocytes) and 800 µL PVP-I product (undiluted, 1:10 or 1:100 dilution). A virus control mixture was also assessed using double-distilled water in place of the test product. After the specified contact time (15, 30 or 60 s), virucidal activity was immediately suppressed by dilution with nine volumes of ice-cold medium (minimal essential medium + 2.0% fetal calf serum) and serially diluted tenfold. Infectivity was determined by

means of end point dilution titration in microtiter plates. Aliquots of 100 µL from each dilution were added to six 200 µL samples of BHK-21 cells. Cultures were examined microscopically for cytopathic effects (CPE) after 8 days of inoculation.

The virus titers were determined using the Spearman–Kärber method [11, 12] and expressed as tissue culture infectious dose 50% ($TCID_{50}$/mL). The virucidal activity was determined by the difference of the logarithmic titer of the virus control minus the logarithmic titer of the test virus ($\Delta \log_{10}$ $TCID_{50}$/mL). This difference is presented as a reduction factor (RF) including its 95% confidence interval (CI). A reduction in virus titer of $\geq$4 $\log_{10}$ (corresponding to an inactivation of $\geq$99.99%) was regarded as evidence of sufficient virucidal activity. The calculation was performed according to EN14476 [10].

Inactivation Assay Using Large Volume Plating (LVP) Method for Verification of Concentration–Contact Time Values with Mers-CoVv

In accordance with EN14476:2013/FprA1:2015, the inactivation tests were conducted once, at 20 ± 1 °C [10]. One part MERS-CoV suspension (100 µL) was mixed with 100 µL of either 0.3 g/L BSA (clean conditions) or 3.0 g/L BSA + 3.0 mL/L erythrocytes (dirty conditions) as the interfering substance. The virus–protein mixture was added to 8 parts (800 µL) of the undiluted test product. After a contact time of 15 s, 20 µL of the test mixture was added to 99.98 mL ice-cold medium. Aliquots (300µL) of the diluted sample were then added to 336 wells containing the indicator cells. The cells were cultivated for 5 days, and then inspected microscopically after 3 and 5 days for virus-induced CPE in cell morphology. Calculations of viral titer (in cases of no virus or low viral count) were as detailed in [8].

RESULTS

Determination of the PVP-I Kinetics in Clean and Dirty Conditions Using MVA

The test concentrations and contact periods were chosen to observe the point at which each test preparation produced efficient virus inactivation. To demonstrate virucidal efficacy, disinfectant and antiseptic products are required to produce a $\log_{10}$ reduction in virus titer of at least 4 [10]. The $\log_{10}$ reduction factors produced by the test products under clean and dirty conditions at each time point are shown in Table 2. With each PVP-I formulation, $\log_{10}$ reduction in viral titer $\geq$4 was demonstrated under clean and dirty conditions after only 15 s with the undiluted and 1:10 dilutions (except for gargle/mouthwash, which, in dirty conditions, required 30 s of exposure at the 1:10 dilution).

Under both clean and dirty conditions, the virucidal activity of the PVP-I products varied with the concentration of available iodine as follows: scrub, 0.75 g/L > 7.5 g/L > 0.075 g/L; cleanser, 0.4 g/L > 4.0 g/L > 0.04 g/L; gargle/mouthwash, 1.0 g/L > 0.1 g/L > 0.01 g/L.

Verification of Concentration–Contact Time Values with MERS-CoV

The titers of MERS-CoV present in the control samples ranged from 6.00 to 6.50 $\log_{10}$ $TCID_{50}$/mL under clean and dirty conditions. MERS-CoV viral titers were reduced between 4.30 and 4.97 $\log_{10}$ $TCID_{50}$/mL after 15 s (Table 2), which corresponds to a reduction in

Table 2 Virucidal activity of PVP-I skin cleanser, surgical scrub and gargle/mouthwash against MVA and MERS-CoV

Test product	Dilution	Log_{10} reduction factor (95% CI[a])							
		Clean conditions[b]				Dirty conditions[b]			
		MERS-CoV	MVA			MERS-CoV	MVA		
		15 s	15 s	30 s	60 s	15 s	15 s	30 s	60 s
PVP-I surgical scrub[c] (7.5 g/L available iodine)	Undiluted	**4.64**	**≥4.00**	**≥4.00**	**≥4.00**	**4.64**	**≥4.17**	**≥4.17**	**≥4.17**
	1:10	n.d.	**≥5.50**	**≥5.50**	**≥5.50**	n.d.	**≥5.67**	**≥5.67**	**≥5.67**
	1:100	n.d.	3.83 (±0.65)	**4.17** (±0.58)	**4.50** (±0.58)	n.d.	1.00 (±0.70)	1.67 (±0.70)	1.83 (±0.71)
PVP-I skin cleanser[c] (4 g/L available iodine)	Undiluted	**4.97**	**≥4.17**	**≥4.17**	**≥4.17**	**4.97**	**≥4.00**	**≥4.00**	**≥4.00**
	1:10	n.d.	**4.50** (±0.54)	**≥4.67**	**≥4.67**	n.d.	**4.33** (±0.56)	**≥4.50**	**≥4.50**
	1:100	n.d.	3.33 (±0.56)	3.67 (±0.47)	3.67 (±0.47)	n.d.	0.33 (±0.56)	1.00 (±0.63)	1.00 (±0.63)
PVP-I gargle and mouthwash (1 g/L available iodine)	Undiluted	**4.30**	**6.50** (±0.45)	**6.50** (±0.45)	**6.50** (±0.45)	**4.30**	**6.50** (±0.45)	**6.50** (±0.45)	**6.50** (±0.45)
	1:10	n.d.	**4.83** (±0.71)	**5.83** (±0.71)	**5.83** (±0.61)	n.d.	3.50 (±0.45)	**4.00** (±0.63)	**4.00** (±0.63)
	1:100	n.d.	0.67 (±0.56)	0.67 (±0.56)	0.67 (±0.70)	n.d.	0.50 (±0.65)	0.67 (±0.70)	1.00 (±0.63)

Results shown in bold indicate virucidal activity (≥4 log_{10} reduction in viral titer)

CI confidence interval, *LVP* large volume plating, *MERS-CoV* Middle East respiratory syndrome coronavirus, *MVA* modified vaccinia virus Ankara, *n.d.* not determined, *PVP-I* povidone iodine, *s* seconds exposure time

[a] No confidence interval available for MERS-CoV (reduction factor calculated with Poissons formula of LVP) or for values expressed as ≥x

[b] Clean conditions: 0.3 g/L bovine serum albumin (BSA); dirty conditions: 3.0 g/L BSA + 3.0 mL/L erythrocytes

[c] MVA data from Ref. [8]

MERS-CoV viral titer of ≥99.99% for all products tested.

DISCUSSION

As is the case for Ebola, MERS-CoV is an enveloped virus with a high biosafety level, for which there is no vaccination, nor any specific antiviral treatment [13, 14]. While infection can remain subclinical—indicating that not all cases may be reported—MERS-CoV more typically causes severe respiratory disease. During the first year following the first reported case, two-thirds of patients suffered severe disease [15], and over a third of reported cases to date have been fatal [4].

Containment of spread has proven achievable in most cases; thus far, no sustained human-to-human transmission has occurred anywhere in the world [4]. However, a lack of awareness among health care workers and the general public, coupled with inadequate prevention and control procedures, can result in outbreaks based on nosocomial infection, as recently observed in the Republic of Korea. Based on the current situation, the World Health Organization (WHO) has issued a number of recommendations [4]. Many of these reflect the lack of understanding of how humans become infected from animal or environmental sources, with particular emphasis on precautions relating to exposure to camels. The other focus is on ensuring that health care facilities adopt appropriate measures to decrease the risk of transmission of the virus from an infected patient to other patients, health care workers and visitors.

A practical measure applicable to both of these issues is the implementation of effective hand hygiene practice. Standard hand hygiene includes either washing hands with soap and water or the use of an alcohol-based hand rub [16]. Randomized, controlled trial data are available to support the effectiveness of PVP-I and alcohol hand rubs over plain soap hand wash for hand decontamination, based on post-hygiene colony-forming unit count [17]. In the context of virucidal activity, PVP-I has demonstrated superiority over ethanol-based sanitizers in inactivating murine norovirus on a modified finger pad test [18]. In an evaluation of the effectiveness of nine different hand sanitizers against feline calicivirus (a surrogate for norovirus), antiseptics containing 10% PVP-I achieved a greater viral reduction rate than any of the alcohol-based sanitizers, non-alcoholic sanitizers or antimicrobial soaps [19]. PVP-I has demonstrated virucidal activity against a range of enveloped and non-enveloped viruses. Perhaps most relevant in the context of the MERS-CoV is the evidence for effective inactivation of the SARS-CoV to below detectable levels within 2 min of exposure [20].

Effective hand hygiene is crucial in minimizing viral transmission from the contaminated hands of an infected individual, either through direct person-to-person contact, or indirectly via contamination of surfaces. However, respiratory viruses are also subject to airborne (particles ≤5 μm in size) or droplet (>5 μm) transmission, in which infected material is released by the infected individual breathing, coughing or sneezing [21]. Gargling represents an effective personal hygiene measure against airborne/droplet transmission, as it can reduce the microbe count at the pharynx [22]. Together with hand washing and mask use, it has been proposed that gargling is one of the three major personal hygiene protection measures against common airborne and droplet-transmitted infections [22]. Specialists advise that the criteria for

selecting mouthwashes should include effectiveness of the antiseptic agent in killing pathogens [22]. Given the strong in vitro virucidal activity of PVP-I demonstrated in this and other studies, gargling/flushing with PVP-I may be an effective measure to disrupt the transmission of respiratory viruses, especially via airborne/droplet transmission or after uptake via the mouth (such as when touching the mouth or food with contaminated hands).

The data reported here indicate rapid virucidal activity of three formulations of PVP-I against both MVA and MERS-CoV. The 15-s exposure time was assessed to study the virucidal kinetics of PVP-I against MVA over time (the minimum contact time for hygienic hand rub and hand wash defined in EN14476:2013 is 30 s [10]), and yet proved sufficient for all three formulations to be effective, against both MVA and MERS-CoV.

MERS-CoV is a harmful enveloped virus and requires high biosafety levels for any investigation. It is not recommended that disinfectants are tested using highly contagious and harmful viruses; thus, model viruses are used. The CEN/TC 216/WG1 committee, which establishes standardized European testing methods and requirements for the antimicrobial efficacy of chemical disinfectants and antiseptics, recently implemented the enveloped MVA as the model virus for the claim 'virucidal active against enveloped viruses for hygienic hand rub and hand wash'. MVA was chosen on the basis of its low biosafety level, its known environmental stability and its practicability for laboratory use [23–25]. On the basis of these practical safety concerns, our study was conducted primarily using MVA with confirmation only in MERS-CoV.

Usually, only low titers of MERS-CoV can be harvested in cell culture, resulting in a range of 5.00–6.50 $\log_{10}$ $TCID_{50}$/mL. To demonstrate at least a 4 $\log_{10}$ reduction in virus titer, it is necessary for test mixtures containing low virus titers to undergo detoxification by molecular sieving, or to use a more sensitive assay such as LVP [26]. In LVP, a high volume of the lowest apparently non-cytotoxic dilution of the inactivation assay test mixture is added to the detector cell line and the cultures are monitored for virus-specific effects. Using this method, larger reductions in virus titer can be shown even at lower viral loads and contact times as short as 15 s can be tested, minimizing the impact of any after effects. LVP offers sufficient sensitivity for reductions in virus titer to be detected even using test products that are highly cytotoxic in cell culture [27].

It should be considered that while the results of this in vitro analysis are a suitable basis for predictions about the virucidal efficacy of PVP-I, they do not provide direct information about the effectiveness of the products in practice. However, taken together with: (1) the emphasis placed by WHO on ensuring suitable levels of hand hygiene; (2) recommendations for gargling with antiseptic mouthwash for the control of common airborne and droplet-transmitted infections [22]; (3) the fact that PVP-I is a product already in use and available within the most affected regions of the world, these data provide strong rationale for the use of PVP-I products for the prevention of infection by MERS-CoV. Improved awareness is needed in the health care setting to ensure effective containment of the spread of this often fatal virus.

CONCLUSION

The outbreak of MERS-CoV in the Republic of Korea is testament to the ongoing risk of healthcare-associated transmission, and

reinforces the need for timely diagnosis and implementation of prevention and control measures. The three PVP-I products tested in this study demonstrated virucidal activity against MVA and MERS-CoV at room temperature, within only 15 s of exposure. This was observed under both clean and dirty conditions. These data are consistent with those from other studies demonstrating the excellent virucidal activity of PVP-I against enveloped viruses.

The data reported here indicate that PVP-I-based hand wash products for potentially contaminated skin, together with PVP-I gargle/mouthwash for reduction of viral load in the oral cavity and the oropharynx, may help to support hygiene measures during outbreaks of respiratory viruses.

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MEg was responsible for the study design and performed the statistical analysis, provided analysis and interpretation of data, and carried out the virucidal tests with MVA. ME carried out the virucidal tests with MERS-CoV and provided analysis and interpretation of data. JZ participated in the original planning and design of the study and contributed to the interpretation of results as well as to the manuscript.

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval to the version to be published.

Disclosures. JZ is an employee of Mundipharma Research GmbH & Co. KG and has no other financial or non-financial competing interests. MEg and ME have nothing to disclose.

Compliance with ethics guidelines. This article does not contain any new studies with human or animal subjects performed by any of the authors.

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